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***FINAL REPORT***

20070020

Potential for using biofuel, crop and animal processing by-products as soil amendments to increase fertility

Funded by: The Agriculture Development Fund

February 2009

Prepared by: University of Saskatchewan (U of S)



**Agriculture Development Fund Project no. 20070020 MIDAS# 000598**

**POTENTIAL FOR USING BIOFUEL, CROP AND ANIMAL PROCESSING  
BY-PRODUCTS AS SOIL AMENDMENTS TO INCREASE FERTILITY:**

**Effect of Soil Amendment with Dehydrated Alfalfa, Dry and Wet Distillers  
Grains, Thin Stillage and Glycerol on Growth of Canola, Soil Properties,  
and Microbial Activity**

**FINAL REPORT**

**January 2009**

**Summary**

Controlled environment experiments were set up in 2008 to evaluate the effect of adding various biofuel and crop processing co-products to soil as organic amendments to increase soil fertility, crop growth, soil organic matter and microbial activity. Amendments evaluated included dehydrated alfalfa, wet and dry distillers grain, thin stillage and glycerol applied at different rates in comparison with urea fertilizer. Canola was grown as the test crop in amended pots, and plant yield, composition and soil properties were measured after five weeks. The same treatments were prepared and used in an incubation experiment in which carbon dioxide, nitrous oxide gas, and nutrient ion release by soil microbial populations was assessed.

The alfalfa, distillers grain and stillage products were found to be effective soil amendments for increasing canola biomass yield. Per unit of nitrogen added, yields were less than that of urea when nitrogen was the only limitation, due to only a portion of the nitrogen in the amendment becoming available over the five week period. However, when nutrients other than nitrogen were limiting, canola dry matter yields with organic amendment approached or exceeded that of urea, due to the ability of the amendments to supply other nutrients in addition to nitrogen. Glycerol, an amendment that only contains carbon, hydrogen and oxygen, was effective in increasing soil organic carbon content, but required supplemental fertilizer to account for nutrient tie-up by microorganisms during decomposition in the soil. The amendments did not have any biologically significant effects on soil chemical parameters measured including soluble metals, pH or salinity. Some initial reduction in germination and emergence of canola plants at the highest rate of distillers grain was observed, the nature of which was not identified.

Application of solid amendments like alfalfa and distillers grain enhanced microbial activity to the greatest extent as revealed in carbon dioxide gas production. Per unit of nitrogen added, urea resulted in the greatest production of nitrous oxide gas and alfalfa the least. The supply rates of nitrate to PRS probes during the incubation were closely related to patterns in nitrous oxide production, indicating that nitrification is likely the main source of nitrous oxide production when organic and inorganic fertilizer amendments are initially added to the soil.

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## PART 1: Controlled Environment Canola Fertility and Growth Experiments

### Background/Introduction

Five controlled environment experiments were set up in the spring of 2008. The intent of the experiments was to evaluate the potential of using products of the processing and biofuel industry in Saskatchewan as soil amendments to improve fertility and plant growth. These products would normally be used as animal feeds. Given the recent escalation in fertilizer costs and the possibility of traditional markets becoming saturated or diminishing, alternative uses for such products need to be explored. One possible use is as a fertilizer. The effects of using dehydrated alfalfa as a nitrogen source for crop growth was first evaluated, as recent issues in production and export of dehydrated alfalfa products in Saskatchewan have led to searching for alternative uses. Then trials were run with dry distillers grains with solubles (DDG), wet distillers grain (WDG), and thin stillage. These are the main by-products of the grain ethanol production process, which is an emerging industry in Western Canada. The alfalfa and ethanol production by-products contain organic matter along with plant macro and micro-nutrients. In biodiesel production, glycerol is the main by-product. The final trial was run with glycerol, a pure carbon source without plant nutrients, of which the main potential benefit is to add to soil organic carbon sequestration as an alternative to burning. There has been little or no work published on the utilization of biofuel co-products as soil amendments.

### Experimental Design

The original proposal entailed the evaluation of three products in the growth chamber: 1) wet distiller's grain, 2) glycerol from biodiesel production and 3) canola meal fractionation by-product combined with alfalfa as a pellet. Owing to industry interest in other ethanol production by-products, including dry distiller's grain and thin stillage liquid from the fermentation as soil amendments, these two by-products were also included in the evaluation. Therefore two separate experiments were added with dry distillers grain and thin stillage, along with the wet distillers grain. The alfalfa products produced by MCN Bioproducts/Canpro Ingredient were only available as a powder, not as a pellet, so powdered forms of two products they produce were evaluated to complement previous work done in 2007 with pelleted forms. We did not include any ash from gasification of animal processing by-products due to lack of availability and biosafety issues.

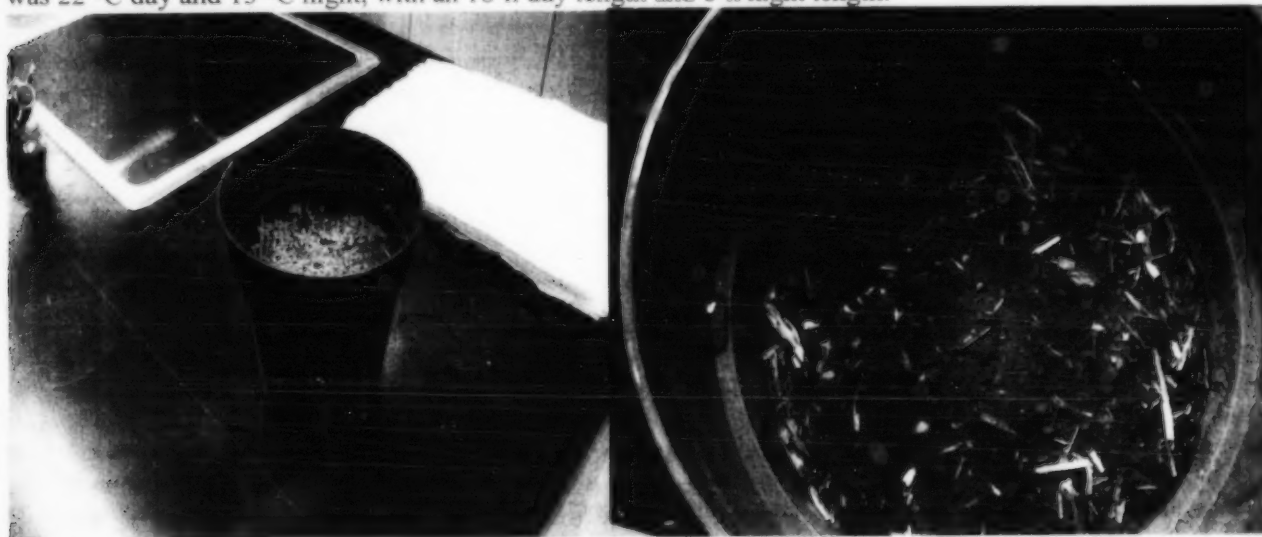
### Alfalfa Experiment:

Two types of alfalfa dried powder were obtained from MCN Bioproducts: "Dehy" and "Sun Cure". They were tested by comparing with urea applied at the same rates of N. The N content is 2.54% for Dehy and 3.34% for Sun Cure, so they represent a relatively concentrated source of N compared to many other organic sources such as fresh cattle manure. The S content is 0.28% for Dehy and 0.45% for Sun Cure. The C:N, C:S ratio is 17:1, 153:1 for the Dehy and 13:1, 95:1 for the Sun Cure. Amendments with C:N and C:S ratios less than 20:1 and 200:1 respectively, result in immediate net mineralization (release) of plant available inorganic N and S upon decomposition. The narrower ratio of the Sun Cure suggests more rapid release of available N and available S from this alfalfa powder source compared to the Dehy product. The experiment used 4 application rates (0, 100, 200 and 400 kg N ha<sup>-1</sup>) with 4 replicates. Four pots were prepared for each treatment. In each pot, 800 g of air-dried soil was weighed out and 200 ml of water was added, followed by application of alfalfa powders or urea on the surface of the soil and then covered with an additional 100 g of soil. A blanket P, K and S solution was then applied at a rate of 25 kg P ha<sup>-1</sup>, 50 kg K ha<sup>-1</sup> and 25 kg S ha<sup>-1</sup>, so as to allow the effect of the alfalfa amendment specifically on nitrogen availability to be revealed. After the fertilized soils in the pots were equilibrated for 48 hours, about 10 canola (*B. napus* cv. Invigor 5020) seeds were placed on the surface of the soil and then covered with another 100 g of soil. The plants were thinned after emergence. Soil moisture was kept at approximately 80% of field capacity in all the treatments and temperature was maintained at room temperature. After

germination, pots were moved to the growth chamber. Temperature in the growth chamber was 22 °C day and 13 °C night, with an 18-h day length and 6-h night length.

#### Distillers Grain Experiment

Two different experiments were conducted with distillers grain: One was with dry distillers grains with solubles (DDG) obtained from the Feed Resource Center at the University of Saskatchewan. The DDG was produced from distillation using a predominately wheat feed stock, but with a small amount of corn also used (<20%). The wet distillers grain (WDG) was obtained from Poundmaker ethanol production facility at Lanigan and did not contain solubles, as the solubles are contained in the thin stillage liquid which was evaluated separately. Urea was applied at the same rates of N as the distillers grains for comparison. The N content is 6.31% for dry distillers grain with solubles and 3.65% for wet distillers grain product, all on a dry basis. The S content is 0.93% for DDG and 1.37% for WDG. The C:N, C:S for DDG is 7:1, 49:1 and 14:1, 36:1 for the WDG. C:N and C:S ratios less than 20:1 and 200:1 respectively, predict immediate net mineralization (release) of plant available inorganic N and S upon decomposition. Based on the narrower C:N, a more rapid release of available N is predicted from DDG than WDG. Application rates for both experiments were the same (0, 100, 200 and 400 kg N ha<sup>-1</sup>) with 4 replicates. Four pots were prepared for each treatment. In each pot, 800 g of air-dried soil were weighed into a pot. The distillers grain products or urea were then mixed with 10g soil and the mixture was spread on the surface of the soil, followed by addition of 200ml deionized water. An additional 100 g of soil was then added to cover the products and the soil were equilibrated for 4 days. Then 10 canola seeds were placed on the soil surface in each pot and an additional 100ml of water was added. The last 100g soil was added on the soil surface to bring the total soil weight to 1000 g for each pot. Soil moisture was kept at approximately 80% of field capacity in all the treatments. After germination, pots were moved to the growth chamber. Temperature in the growth chamber was 22 °C day and 13 °C night, with an 18-h day length and 6-h night length.



Pot with wet distillers grain added to soil (left), followed by addition of another layer of soil and canola seeding.

#### Thin Stillage Experiment

During ethanol production, starch is converted to ethanol and the other constituents of the grain become co-products, including DDG and WDG. One other co-product is thin stillage (TS), a liquid by-product of fermentation in the ethanol production process. Thin stillage can be used as a nutrient-containing water source for livestock, but may have other potential uses as well. There is currently no information as to how thin stillage

from wheat based ethanol production applied to soil might affect soil organic matter, nutrient availability, and biological activity. The residual liquid (soluble) thin stillage fraction after distillation and separation contains the vitamins, minerals, fatty acids, fiber, yeast cells, enzymes, unfermented sugars, soluble amino acids, organic acids, nutrients, and proteins of the parent grain which are concentrated by removal of starch.

Thin stillage samples were obtained from Pound-Maker Agventures ethanol plant (Lanigan, SK) and stored at 4°C before analysis. This thin stillage was analyzed and a list of these properties and their concentration is presented below. The thin stillage contains about 50 lbs of total N/1000 gallons, similar to some **liquid swine manure slurries**. About 20% of the Total-N is comprised of immediately plant available ammonium. The Total-N to Total-P ratio is about 5:1, being wider than many manure sources. The N/S ratio is about 5:1, in line with relative requirements of canola for N and S. The thin stillage also contains many micro-nutrients necessary for plant growth and development. The thin stillage has an acidic pH of 3.8, which is likely due to the presence of organic acids. Thin stillage has very high water content (92%) and includes water soluble materials including protein, unconverted starch and sugars.

#### Analysis of Thin Stillage (TS) Used in Controlled Environment Experiments.

Element	Result	Units
NH <sub>4</sub> -N	9	lb/1000gal
Total-N	47	lb/1000gal
P	9	lb/1000gal
K	11	lb/1000gal
S	6	lb/1000gal
Na	4	lb/1000gal
Ca	2	lb/1000gal
Mg	4	lb/1000gal
Cu	<0.01	lb/1000gal
Fe	0.06	lb/1000gal
Mn	0.04	lb/1000gal
Zn	0.07	lb/1000gal
Total Solids	7.5	%
% Moisture	92.5	%
pH	3.8	pH
EC	5160	μS cm <sup>-1</sup>

The thin stillage used in the growth chamber study was collected and stored at 4°C. Three thin stillage application rates were used in the growth chamber study; 10.64, 21.28, and 42.55 g/pot, equivalent to 0, 200, 400 kg N ha<sup>-1</sup> respectively (0, 50, 100, and 200 μg N g soil<sup>-1</sup>), as for the other experiments. Three urea rates 0.108, 0.216, and 0.432 g/pot added to deionized water were used in the growth chamber study, equivalent to 0, 200, and 400 kg N ha<sup>-1</sup> respectively. In each pot, 800 g of air-dried soil were weighed into a pot. The TS or urea were then spread on the surface of the soil, followed by addition of 200ml deionized water. An additional 100 g of soil was then added to cover the products and the soil were equilibrated for 4 days. Then 10 canola seeds were placed on the soil surface in each pot and an additional 100ml of water was added. The last 100g of soil was added on the soil surface to bring the total soil weight to 1000 g for each pot. Soil moisture was kept at approximately 80% of



field capacity in all the treatments. Temperature in the growth chamber was 22 °C day and 13 °C night, with an 18-h day length and 6-h night length.

#### *Glycerol Experiment:*

The glycerol experiment was set up under the same controlled environment conditions as the others. However, since the glycerol only contains carbon, the rates of application were selected based on carbon. The glycerol chosen for use in the experiment was glycerol that was stripped of  $\text{CH}_3\text{OH}$ , and originated from biodiesel production in Saskatchewan by Milligan Biotech. Four application rates were tested at 0, 100, 1,000, and 10,000  $\text{kg ha}^{-1}$  as glycerol; equivalent to 0, 40, 400 and 4000  $\text{kg C ha}^{-1}$ , given a C content of the glycerol of 40% C by weight. These rates of glycerol were applied alone, and with the equivalent of 150  $\mu\text{g N g}^{-1}$  soil (300  $\text{kg N ha}^{-1}$ ) of urea added. The experiment was a completely randomized design with four replicates. The different amounts of glycerol, or glycerol plus urea required for each pot were dissolved and diluted in water to 200 ml and the solution was added to pots containing 850 g soil. More water was then added to bring the soil to near field capacity. After 24 hours of equilibration, ten canola seeds were seeded, and then covered with an additional 150 g of soil. Soil moisture was kept at approximately 80% of field capacity in all the treatments. After germination, pots were moved to the growth chamber. As for the other experiments, temperature in the growth chamber was 22 °C day and 13 °C night, with an 18-h day length and 6-h night length.

#### **Plant and Soil Analysis**

In both experiments, canola seeds were almost completely germinated and emerged within 3 to 5 days after seeding in all the treatments. After emergence, seedlings were thinned to 3 plants per pot. The pots were completely randomized and rotated each week. Canola plants were grown for five weeks, and then the above ground biomass was harvested. After harvesting, plants were dried at 50°C, and weighed for dry matter yield determination. The samples were then ground and digested in a sulfuric acid-hydrogen peroxide mixture using a temperature-controlled digestion block. Concentrations of elements in the digest were determined by colorimetry and spectroscopy. Plant nutrient uptake was calculated by multiplying the yield by the concentration in the tissue and is expressed as milligrams of nutrient taken up per kilogram of soil.

The soil used in the experiments was collected from the 0-15cm depth of a wheat stubble field near Central Butte, SK in the fall of 2007. The soil is mapped as an Orthic Brown Chernozem of the Ardill Association. About 200 kg of soil was collected from the field using a front-end loader. After collection, the soil was mixed thoroughly in a soil mixer and stored before use. A sample of the soil used in preparation of pots for each experiment was collected and analyzed. The initial properties of the soil used in each experiment are shown below. The mixing resulted in a reasonably uniform soil, as evident by similar analytical results for each of the soils used to prepare pots. At the end of the canola growth period, the soil from each pot was removed and prepared for analysis. Sub-samples of soil were collected from the mixed soil, and then air-dried, crushed, and passed through a 2-mm sieve and stored at room temperature. Electrical conductivity (EC) and pH were measured using 1:1 soil:water suspension. Organic C (OC) was measured using Leco carbon analyzer. Soil total N and P concentrations were determined by sulfuric acid peroxide digest. Plant available inorganic N ( $\text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ ) was extracted with 2 M KCl,  $\text{SO}_4\text{-S}$  by 0.01M  $\text{CaCl}_2$ , and available P and K were extracted using the Modified Kelowna procedure. Soil available Cu, Zn and Cd were extracted by AB-DTPA.

Initial properties of the soil used in the controlled environment experiments.

Amendment Source	Total N mg/g	Total P mg/g	OC %	pH	EC mS cm <sup>-1</sup>	NO <sub>3</sub> <sup>-</sup> N ug/g	NH <sub>4</sub> <sup>-</sup> N ug/g	P ug/g	K ug/g	SO <sub>4</sub> <sup>-</sup> S ug/g	Cu ug/g	Zn ug/g	Cd ug/g
Alfalfa	1.12	0.48	1.92	8.04	0.29	1.7	6.9	5.6	591	14.3	0.45	6.9	0.052
Dry Distiller	1.18	0.50	1.94	8.00	0.30	1.7	5.5	6.0	609	13.5	0.41	4.1	0.066
Wet Distiller	1.05	0.49	1.87	7.97	0.29	1.7	3.6	5.0	601	11.6	0.49	4.6	0.072
Thin Stillage	1.17	0.50	1.90	8.00	0.30	2.4	6.7	4.2	587	12.0	0.50	4.3	0.050
Glycerol	1.17	0.49	1.85	7.96	0.29	1.7	5.3	6.3	654	15.4	0.43	3.8	0.063

## Results and Discussion

### Alfalfa Experiment

The alfalfa amendments produced canola dry matter yields and plant nitrogen uptake at equivalent rates of added N that were about 30% to 50% of that obtained for urea (Table 1.1). Since all treatments received a basal application of P,K and S, differences between the alfalfa and urea amendments are likely mainly due to differences in N availability, with an estimated availability of N from the alfalfa treatment over the five week period of about 30 to 50% of that from commercial urea. Other possible beneficial effects on plant growth may arise from the organic matter added in the alfalfa, such as increased microbial activity and improved soil tilth. These may also contribute to yield benefit. The highest dry matter yields were obtained at the 400 kg N ha<sup>-1</sup> rate for all amendments. It is important to note that the responses observed to rates of N in pot experiments occur at generally higher rates than in the field. This is because the root volume is greatly restricted in the pots such that higher concentrations per unit weight or unit volume of soil are needed to see the same yield response. Although both the "Dehy" and "SunCure" amendments were made at the same rates of nitrogen, the higher yields and N uptake from the "SunCure" can be attributed to the lower C:N ratio of this particular alfalfa dehy product. Lower C:N ratios of organic amendments are associated with increased rates of available N release by mineralization. Therefore, there is a need to consider not only the total concentration of N in the amendment, but also the C:N ratio when making predictions of N availability from organic amendments. Uptake of other nutrients including P, K and the micronutrient Zn increased with addition of alfalfa or urea, in line with increased N availability and plant demand for other nutrients. Uptake of the non-functional element cadmium also increased with amendment addition, as fertilization will increase root growth and uptake potential for both functional nutrient and non-functional, non-nutrient elements. The high rate of urea resulted in the highest yield and highest Cd uptake.



Table 1.1 Properties of canola plants grown in alfalfa powder and urea amended soil.

Fertilizer Rate	Yield	Uptake				
		N	P	K	Zn	Cd
kg ha <sup>-1</sup>	g/pot	mg/kg	mg/kg	mg/kg	ug/kg	ug/kg
0	0.63g	8.4h	2.19f	11.3g	48.6bc	206e
100 (Dehy)	1.20fg	11.3h	3.70e	23.1f	40.7c	361de
200 (Dehy)	1.83e	17.5gf	5.14d	32.5e	59.4bc	559b
400 (Dehy)	3.16d	29.4d	8.47b	55.9d	97.8bc	519bcd
100 (Sun Cure)	1.63ef	13.3h	4.53de	30.5ef	86.7bc	372de
200 (Sun Cure)	2.66d	22.6ef	6.57c	54.4d	60.0bc	409cd
400 (Sun Cure)	4.22c	42.0c	10.98a	94.1c	167.3ab	598b
100 (Urea)	2.92d	23.6d	6.74c	57.5d	46.5bc	365de
200 (Urea)	5.33b	54.8b	10.38a	106.7b	123.8bc	617b
400 (Urea)	6.83a	104.1a	10.83a	192.5a	281.2a	1302a

Numbers in a column followed by the same letter are not significantly different at  $P < 0.10$ .

The properties of the soil before and after five weeks of canola growth with the alfalfa and urea amendments are shown in Table 1.2. Compared to the soil before amendment addition and canola seeding and growth, the soil at the end of the experiment had available nitrogen levels that were only slightly elevated. There was also relatively little difference among the amendment treatments. The high rates of the alfalfa amendments generally had higher residual inorganic N than the other treatments. This may reflect some additional mineralization of organic N from the alfalfa amendments towards the end of the experiment. Residual available P, K and S and total P were generally higher in the alfalfa amended treatments compared to the urea treatments, indicating that the alfalfa amendment also contributes significantly to P, K and S fertility, although the effect of this contribution on yield was masked in this experiment by the addition of basal P, K and S to all treatments. All the amendments tended to increase total N content with increasing rate of addition to a similar magnitude, not surprising given that the same rates of total N were used. The amendments had little or no influence on soil pH. Any effects on EC (salinity) were also small. Organic carbon concentrations tended to be slightly higher in alfalfa amended treatments than for urea, especially at high rates of addition, although the differences were not great, and not much different from the control. It is anticipated that a single application of the alfalfa amendment would have relatively little effect on soil organic carbon content. Amendment with alfalfa or urea tended to have very little biologically significant effect on concentrations of Cu, Zn and Cd in the soil at the end of the experiment, although effects were sometimes statistically significant. There was a tendency for the amendments to be lower in residual soil micronutrient content, perhaps because of little added in the residue and enhanced plant growth resulting in greater removal.

Table 1.2 Soil properties in alfalfa powder amendment trial before and after canola harvest.

Fertilizer Rate N kg ha <sup>-1</sup>	NO <sub>3</sub> -N ug/g	NH <sub>4</sub> -N ug/g	P ug/g	K ug/g	SO <sub>4</sub> -S ug/g	Total N mg/g	Total P mg/g	pH	EC mS cm <sup>-1</sup>	OC %	Cu ug/g	Zn ug/g	Cd ug/g	
Before seeding	1.7	6.9	5.6	591	14.3	1.12	0.48	8.04	0.29	1.92	0.45	6.9	0.052	
After harvest														
0	2.1cd	5.7b	13.6bc	667bc	25.2a	1.38c	0.52b	7.86b	0.36a	1.86bcd	0.53a	4.8a	0.049bcd	
100 (Dehy)	3.1bc	4.9c	21.7a	797a	24.4a	1.39bc	0.53b	8.00a	0.35ab	1.84cd	0.43b	4.4b	0.046cde	
200 (Dehy)	3.0bc	4.5c	16.2b	718ab	19.3b	1.45abc	0.59a	8.03a	0.34abc	1.98ab	0.40bc	4.6b	0.055a	
400 (Dehy)	4.0ab	6.8a	15.4b	745ab	18.7b	1.52a	0.53b	8.02a	0.34abc	2.02a	0.30c	4.4b	0.050abcd	
100 (Sun Cure)	2.2cd	4.5c	15.0b	673bc	21.6ab	1.38bc	0.53ab	8.04a	0.32bc	1.85bc	0.37bcd	4.8b	0.046de	
200 (Sun Cure)	1.3d	4.6c	12.7bc	674bc	19.4b	1.46ab	0.54ab	8.01a	0.31cd	1.96abc	0.33de	4.8b	0.050abcd	
400 (Sun Cure)	4.6a	6.9a	d	693bc	19.1b	1.52a	0.53b	8.05a	0.34abc	1.99ab	0.39bcd	7.7a	0.054ab	
100 (Urea)	1.3d	4.8c	9.9cd	600cd	18.2b	1.43bc	0.51b	8.04a	0.28de	1.78d	0.40bcd	5.6ab	0.044e	
200 (Urea)	2.3cd	4.8c	7.6d	536de	12.9c	1.42bc	0.51b	8.06a	0.26e	1.89abc	d	0.34cde	5.5ab	0.046de
400 (Urea)	1.4d	6.6a	7.3d	456e	10.0c	1.52a	0.45c	8.06a	0.21f	1.85cd	0.36cde	6.0ab	0.051abc	

Numbers in a column followed by the same letter are not significantly different at  $P < 0.10$ .

### Distillers Grain Experiments

Two separate experiments were run with distillers grain: one with dry distillers grain (DDG) with solubles in comparison to urea, and another with wet distillers grain (WDG) without solubles. The distillers grains were from different origin and sources, so the effects of the solubles cannot be factored out in this study. The DDG produced very good dry matter yield responses of canola (Table 2.1) after five weeks, and the responses at equivalent rates of total N added as DDG were not significantly different, or in the case of the high rate, greater than for urea. One important observation made with both wet and dry distillers grain in this experiment and other preliminary experiments we conducted in the growth chamber with canola, was apparent reduced germination and emergence of the canola seedlings at the high rate of DDG or WDG addition (400 kg N ha<sup>-1</sup>). The appearance of the canola plants was similar to plants affected by too much fertilizer placed in the seed-row. This does not appear to be related to a salt effect however, as the E.C. was low for all DDG and WDG treatments measured five weeks after addition. The nature of this inhibition deserves further attention and is a cautionary if high rates of DG are to be applied in the field. It is also not known if the inhibition might extend to other crops as well. However, it was observed that the canola plants recovered rather quickly and went on to do quite well at the high rates of addition as shown in Table 2.1.

At equivalent rates of added N, the N uptake by the canola grown with DDG was about 80% to 90% of that observed for urea, despite similar or higher yields. As for any organic amendment, not all the N was made available for plant uptake over the five week period of high demand. However, since basal application of P, K, and S was not made in these experiments (unlike the alfalfa experiment), it seems that the contribution of other nutrients like P and also S by the distiller's grain resulted in equivalent or higher yields in the DDG treatments. This is evident in the greater uptake of macronutrients in the DDG treatments compared to urea (Table 2.2). There was no significant effect of treatment on Zn. The uptake of Cd significantly increased with DDG or Urea rate, but there was no significant difference between DDG or urea at the same rate.

Table 2.1. Properties of canola plants grown in dried distillers grain (DDG) with solubles and urea amended soil.

Fertilizer Rate	Yield	Uptake				
		N	P	K	Zn	Cd
kg ha <sup>-1</sup>	g/pot	mg/kg	mg/kg	mg/kg	ug/kg	ug/kg
0	0.74d	7.86f	2.65e	14.4e	40.9a	412 d
100 DDG	2.05c	15.42e	4.50c	40.5d	100.5a	436 d
200 DDG	3.30b	23.77d	5.60b	53.3bc	166.5a	598 bc
400 DDG	5.21a	49.08b	9.67a	101.7a	114.7a	818 a
100 (Urea)	2.37c	17.88e	3.51d	41.2d	154.5a	479 cd
200 (Urea)	3.13b	33.55c	3.51d	46.0cd	217.2a	716 ab
400 (Urea)	3.02b	61.40a	2.61e	56.9b	185.2a	770 a

Numbers in a column followed by the same letter are not significantly different at  $P < 0.10$ .

Canola grown in the wet distillers grain experiment (Table 2.2) tended to follow similar patterns to that grown with dry distillers grain. Of note is that for comparison with the urea treatments in the two experiments, the yields and N uptake with the WDG tended to be less in comparison with urea, versus DDG in comparison with urea. This is likely a consequence of the same amount of total N added but a wider C:N ratio in the WDG, which would result in a lower rate of N release over the 5 week period in comparison to DDG. It is likely that the solubles that are added back to the DDG in the drying process contribute low molecular weight organic nitrogen compounds that have low C:N ratios and are readily mineralized by the soil microbial population. Effects of WDG on other nutrients for canola were similar to DDG.

Table 2.2. Properties of canola plants grown in wet distillers grain (WDG) and urea amended soil

Fertilizer Rate	Yield	Uptake				
		N	P	K	Zn	Cd
kg ha <sup>-1</sup>	g/pot	mg/kg	mg/kg	mg/kg	ug/kg	ug/kg
0	0.51e	4.92e	1.56e	8.82e	19.6e	260.8d
100 WDG	1.02d	6.49de	1.99de	18.77d	19.4e	216.3d
200 WDG	1.44c	10.68bc	2.74bc	26.88c	34.1de	297.7d
400 WDG	2.11a	36.57b	4.38a	59.94a	59.9bc	442.4c
100 (Urea)	1.65bc	14.10c	2.47cd	27.41c	42.8cd	402.0c
200 (Urea)	1.68bc	38.05b	2.53cd	51.06b	75.2ab	602.1b
400 (Urea)	1.95ab	59.38a	3.16b	62.49a	84.2a	775.7a

Numbers in a column followed by the same letter are not significantly different at  $P < 0.10$ .

The DDG and WDG treatments (Tables 2.3 and 2.4) all had low content of plant available inorganic N at the end of the experiments. The high rates of urea had elevated nitrate nitrogen content, indicating some unused fertilizer urea N that was added at these rates. The soil extractable P and K contents were not greatly altered, comparing initial content to those measured at the end after 5 weeks of canola growth. Treatment also had relatively little effect, but with DDG and WDG amendments generally resulting in slightly although not always significantly higher residual extractable P and K. Soil sulfate levels appeared to be little affected by treatment.

Table 2.3 Soil properties in dried distillers grain amendment trial before and after canola harvest.

Fertilizer Rate N kg ha <sup>-1</sup>	NO <sub>3</sub> -N ug/g	NH <sub>4</sub> -N ug/g	P ug/g	K ug/g	SO <sub>4</sub> -S ug/g	Total N mg/g	Total P mg/g	pH	EC mS cm <sup>-1</sup>	OC %	Cu ug/g	Zn ug/g	Cd ug/g
Before seeding	1.7	5.5	6.0	609	13.5	1.18	0.50	8.00	0.30	1.94	0.41	4.1	0.066
After harvest													
0 DDG	2.2b	9.6a	5.7a	626a	9.1a	1.04d	0.49c	8.08ab	0.28b	1.88a	0.47a	2.9b	0.071a
100 DDG	1.9b	8.6a	5.0b	589b	8.9a	1.07cd	0.49c	8.05b	0.26c	1.86a	0.42a	3.4a	0.070a
200 DDG	1.3b	4.1c	4.7bc	570b	8.6a	1.16abc	0.50bc	8.08ab	0.25cd	1.87a	0.48a	2.9b	0.070a
400 DDG	1.3b	5.5b	5.1b	532c	9.0a	1.21ab	0.53a	8.07ab	0.24de	1.94a	0.38a	2.9b	0.065a
100 (Urea)	1.5b	5.6b	4.3c	564b	9.0a	1.14bcd	0.52ab	8.10ab	0.24de	1.89a	0.45a	3.0ab	0.069a
200 (Urea)	2.4b	5.0bc	4.3c	567b	8.9a	1.15abcd	0.50bc	8.06ab	0.23e	1.88a	0.44a	2.9b	0.069a
400 (Urea)	35.9a	6.0b	4.5bc	569b	8.9a	1.27a	0.49c	7.99c	0.35a	1.92a	0.46a	2.8b	0.071a

Numbers in a column followed by the same letter are not significantly different at  $P < 0.10$ .

Consistent with the experiment for alfalfa powder, the total N levels were elevated to about the same extent for the distillers grain and urea amendments, with the highest rates of either one producing significantly higher total soil N contents. This residual N appears to exist mainly in the organic form and may be anticipated to contribute to increased N availability to subsequent crops. The effects of the distillers grain amendments on total P content compared to urea were small and often not significant. The effects on soil pH were small and variable. Effects on salinity as revealed in E.C. measurements were also generally non significant for DDG and WDG, except that the highest urea rate treatment (400 kg N ha<sup>-1</sup>) generally had a slightly elevated EC compared to all other treatments and the initial soil. As for the alfalfa amendments, effects of DDG and WDG on soil organic carbon were small and often not significant. Effects on soil residual extractable copper, zinc and cadmium concentrations in the soil were also small and with few significant differences among treatments. These results indicate that similar to alfalfa, DDG and WDG amendment does not appear to be associated with any significant enhancement of crop functional and non-functional metal uptake or accumulation in soil compared to commercial fertilizer.

Table 2.4 Soil properties in wet distillers grain amendment trial before and after canola harvest.

Fertilizer Rate N kg ha <sup>-1</sup>	NO <sub>3</sub> -N ug/g	NH <sub>4</sub> -N ug/g	P ug/g	K ug/g	SO <sub>4</sub> -S ug/g	Total N mg/g	Total P mg/g	pH	EC mS cm <sup>-1</sup>	OC %	Cu ug/g	Zn ug/g	Cd ug/g
Before seeding	1.7	3.6	5.0	601	11.6	1.05	0.49	7.97	0.29	1.87	0.49	4.6	0.072
After harvest													
0	1.7bc	4.8ab	5.4b	628a	5.4bc	1.10d	0.52a	8.08a	0.27b	1.88bc	0.44a	3.7a	0.061b
100 WDG	1.1c	4.9ab	5.6b	623a	4.9cd	1.22abc	0.51a	8.01b	0.27b	1.95ab	0.46a	3.2b	0.068ab
200 WDG	1.2c	5.2a	5.6b	619a	3.9cd	1.16bcd	0.55a	8.03bc	0.27b	1.93ab	0.46a	2.9bc	0.069ab
400 WDG	1.8c	3.9c	7.1a	592b	8.7a	1.29a	0.55a	7.96c	0.27b	1.98a	0.46a	3.0bc	0.069ab
100 (Urea)	1.1c	5.3a	4.6b	588b	3.5d	1.14dc	0.51a	7.99b	0.24b	1.92abc	0.46a	3.0bc	0.074a
200 (Urea)	15.2b	5.4a	5.2b	593b	4.2cd	1.08d	0.50a	7.96bc	0.29b	1.84c	0.44a	2.8c	0.073a
400 (Urea)	83.6a	4.2bc	4.8b	556c	6.5b	1.24ab	0.51a	7.84d	0.57a	1.93ab	0.45a	3.0bc	0.071a

Numbers in a column followed by the same letter are not significantly different at  $P < 0.10$ .

### Thin Stillage Experiment

Data from the growth chamber study indicate that thin stillage treatments significantly affected canola dry matter production (Table 3.1), and that plant growth was greatly increased when thin stillage was applied. The dry

matter weight (canola biomass) showed a positive response to increasing rates of thin stillage and urea. All thin stillage treatments applied to the soil increased canola dry matter production significantly compared to the unfertilized, unamended control. At equivalent rates of applied N, the biomass yield of canola on thin stillage was similar to urea, especially at the low and medium application rates. The similar yields, N concentration and uptake in canola when equivalent rates of total N as thin stillage and urea were added indicate high availability of N contained in thin stillage, with greater than one half of the N added becoming available for uptake over the five week period. Even when plants were grown in pots treated with the equivalent of 400 kg N ha<sup>-1</sup>, significant increases in canola dry matter yield were observed with the TS. The highest yield was obtained where thin stillage at 400 kg N ha<sup>-1</sup> was applied. Some toxic effects of the high rate of urea were evident, with a lower yield at 400 kg N ha<sup>-1</sup> compared to 200 kg N ha<sup>-1</sup> of urea. This appears to be at least partly related to a nitrogen induced sulfur deficiency in the urea treatments, as the sulfur concentrations in the plant ranged from 0.25% S to 0.45% S in the thin stillage treatments, while the S concentrations in the urea treatments were ten times lower: only 0.038% S to 0.058% S.

The high application rates (400 kg N ha<sup>-1</sup>) of thin stillage did not have a harmful effect on crop growth, and the thin stillage appears to be an effective source of both nitrogen and sulfur, as suggested by its analysis. Increasing N uptake resulting from increasing N fertility is evident in plant tissue samples having increasing N concentration. Significantly higher plant P tissue concentration and P uptake in thin stillage compared to urea amended soil indicates that the thin stillage is an effective P source. Thin stillage application increased the K concentration and uptake in canola. Total Zn concentration and uptake in aboveground biomass was lower in thin stillage treatments when compared to urea. Although the thin stillage adds some Zn, the Zn uptake by the plant is reduced at the low rate. This is possibly related to the high P added, as high soil P can induce Zn deficiency. Total plant Cd concentration (data not shown) was highest in control and was reduced in thin stillage and urea applications, likely as a result of growth dilution. However, total uptake as calculated from the yield and Cd concentration, increased with addition of TS or urea as a result of growth response to amendment. The thin stillage does not appear to increase uptake potential of these metals in canola plant tissue compared to commercial fertilizer.

Table 3.1 Properties of canola plants grown with thin stillage (TS) and urea amended soil.

Fertilizer Rate	Yield	Uptake				
		N	P	K	Zn	Cd
kg ha <sup>-1</sup>	g/pot	mg/kg	mg/kg	mg/kg	ug/kg	ug/kg
0	0.59f	6.4f	1.92f	9.2f	21.6c	409d
100 TS	1.84e	16.0e	4.43c	25.2e	43.2c	446d
200 TS	3.11b	25.2d	6.94b	53.5b	87.6ab	501cd
400 TS	4.97a	46.5b	10.9a	108.4a	101.8ab	876a
100 (Urea)	2.22d	17.4e	2.98d	30.4de	79.8b	397d
200 (Urea)	3.11b	36.1c	2.58de	42.7c	119.8a	614c
400 (Urea)	2.63c	66.2a	2.42e	35.9d	90.5ab	743b

Numbers in a column followed by the same letter are not significantly different at P<0.10

Similar to alfalfa powder and the DDG and WDG amendments, thin stillage added N, P, S, K, Ca, Mg, Fe, Na, Mn, Zn, Cu, and other elements, with potential to supplement plant growth if deficient in the soil. The soil used in these studies had low N availability and low P availability. After five weeks of canola growth, concentrations of soil Total-N were higher in the treatments that received the high rate of thin stillage or urea. Concentrations of Total-P were also increased slightly. The soil NO<sub>3</sub>-N, and NH<sub>4</sub>-N were all low with the exception of the high urea



treatment, where residual nitrate levels were much higher, indicating residual urea N left at the end of the experiment. Extractable available P, K and S levels were slightly higher in the thin stillage treatments than the urea treatment, reflecting the contribution of the thin stillage to the available pools of these nutrients in the soil. Because it contains many nutrients that are required for plant growth, thin stillage did not deplete the soil concentration to as great an extent. Soil organic carbon was slightly but significantly increased over the unamended control by application of thin stillage, suggesting that some of the organic carbon added as thin stillage is sequestered in the soil. However, the thin stillage organic matter content is low and likely would decompose quickly.

In this study, there were no significant differences among treatments in soil residual zinc concentration. Studies have shown that the use of N fertilizer can increase plant Cd uptake, as was shown in the higher Cd concentration in plant tissue in urea treatments compared to thin stillage application. Residual soil extractable Cd concentrations were all low and generally not affected by treatment. It appears that the thin stillage does not appreciably alter the soil chemical or physical environment such that any increase in solubility and availability of indigenous heavy metals would occur. All treatments had similar soil pH values that were not significantly different from the control or the value for the soil at the start of the experiment. A pH of 8.0 suggests that this soil may have free  $\text{CaCO}_3$  and has a higher buffering capacity against pH change. High pH can decrease the uptake of Cu, Fe, Mn, Zn and reduce the amount of exchangeable Al. Only the high rate of urea produced a small but significant increase in EC. In this study, after one application, the thin stillage does not appear to increase salinity in soil.

Table 3.2 Soil properties in thin stillage and urea amendment trial before and after canola harvest.

Fertilizer Rate N kg ha <sup>-1</sup>	NO <sub>3</sub> -N ug/g	NH <sub>4</sub> -N ug/g	P ug/g	K ug/g	SO <sub>4</sub> -S ug/g	Total N mg/g	Total P mg/g	pH	EC mS cm <sup>-1</sup>	OC %	Cu ug/g	Zn ug/g	Cd ug/g
Before seeding	2.4	6.7	4.2	587	12.0	1.17	0.50	8.0	0.30	1.90	0.50	4.3	0.050
After harvest													
0	2.2b	6.4ab	4.2c	594a	9.5bc	1.19b	0.46b	8.0a	0.25b	1.92b	0.47a	2.83a	0.057c
100 TS	2.1b	6.3ab	4.3c	605a	10.5ab	1.23a	0.49ab	8.0a	0.26b	1.95ab	0.39b	2.90a	0.067b
200 TS	2.0b	5.0cd	4.8b	599a	11.3a	1.22ab	0.44bc	8.0a	0.25b	1.99a	0.39b	2.97a	0.066b
400 TS	2.6b	6.2ab	5.9a	595a	10.9ab	1.26a	0.51a	8.0a	0.25b	1.96ab	0.49a	2.79a	0.057c
100 (Urea)	2.3b	5.5c	3.5d	586b	10.0ab	1.19b	0.43bc	8.1a	0.23c	1.91b	0.40b	2.98a	0.073a
200 (Urea)	2.5b	4.8d	3.7d	570bc	9.3c	1.21ab	0.41c	8.1a	0.22c	1.94ab	0.41b	2.95a	0.057c
400 (Urea)	20.6a	6.8ab	3.7d	566c	8.4c	1.26a	0.51a	8.0a	0.32a	1.90b	0.45ab	2.94a	0.056c

Numbers in a column followed by the same letter are not significantly different at  $P < 0.10$ .

### Glycerol Experiment

Unlike the other amendments evaluated, the glycerol is a hydrocarbon comprised of carbon, hydrogen and oxygen ( $\text{C}_3\text{H}_8\text{O}_3$ ), and contains no appreciable quantities of mineral nutrient elements. Therefore, following addition to soil, when the glycerol undergoes microbial decomposition available nutrients in the soil will be immobilized by the microbial population in order to carry out the decomposition and production of microbial biomass. This immobilization will reduce the supply of available nutrient to plants and subsequently reduce plant growth. This immobilization phenomena is evident in the reduction in yield and N uptake by the canola at the 1000 and 10,000 kg glycerol ha<sup>-1</sup> rates compared to the unfertilized control (Table 4.1). Uptake of other elements was reduced accordingly due to immobilization and reduced plant demand due to reduction in available N. Evidence that N immobilization is a major factor reducing yield in the glycerol amendment treatments is found in the significantly higher dry matter yields when the glycerol was supplemented with 300 kg N ha<sup>-1</sup> of urea

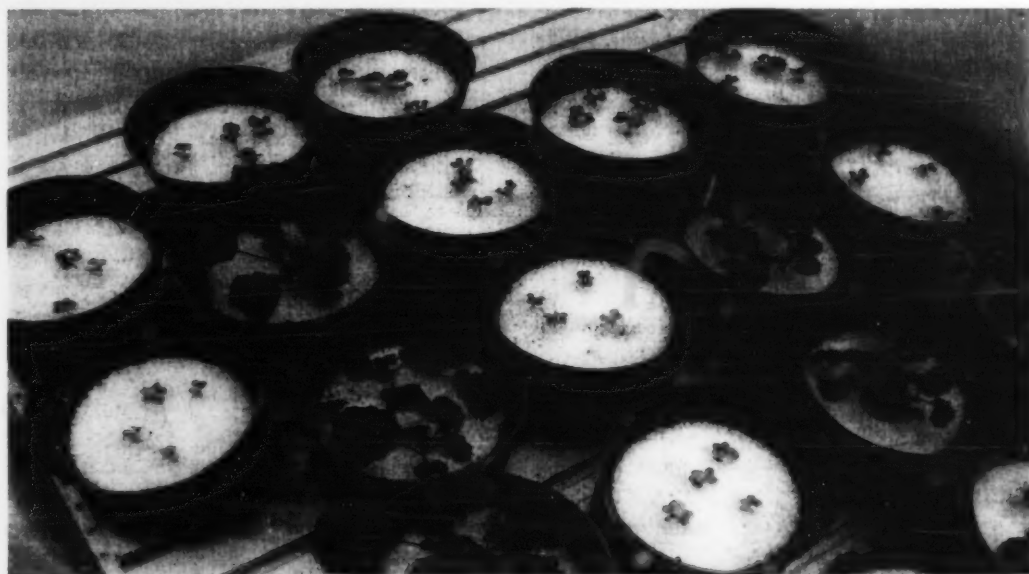


fertilizer. At the 100 and 1000 rates of glycerol addition, the urea was able to maintain yield and nitrogen uptake compared to the urea only fertilized control. However, at the 10000 kg glycerol ha<sup>-1</sup> rate, the yield and N uptake was reduced, likely due to the high immobilization induced by the addition of a very large amount of carbon substrate. Therefore, it would appear that in order to provide sufficient N for crop production at such a high rate of glycerol addition (10000 kg glycerol ha<sup>-1</sup> or 4000 kg C ha<sup>-1</sup>), more than 300 kg N ha of N fertilizer and perhaps P fertilizer would have to be added to both satisfy the requirements of the decomposing microorganisms as well as the plant demand.

Table 4.1 Properties of canola plants grown in glycerol and glycerol + urea amended soil.

Glycerol Rate kg ha <sup>-1</sup>	Yield g/pot	Uptake				
		N mg/kg	P mg/kg	K mg/kg	Zn ug/kg	Cd ug/kg
0	0.39cd	2.52c	0.99cd	6.88bc	15.21d	178.1c
100	0.43c	3.01c	1.15bc	7.01bc	22.19d	206.3c
1,000	0.24cd	1.80c	0.68de	3.50c	11.54d	152.8cd
10,000	0.19d	0.87c	0.49e	3.41c	11.43d	74.48d
0 + 300 (Urea)	2.82a	46.38a	3.16a	63.19a	76.60bc	834.3a
100 + 300 (Urea)	2.66a	46.70a	3.08a	51.03a	96.37ab	811.4a
1,000 + 300 (Urea)	2.81a	44.11a	3.02a	50.38a	118.05a	849.7a
10,000 + 300 (Urea)	1.33b	17.20b	1.46b	19.37b	44.72cd	525.4b

Numbers in a column followed by the same letter are not significantly different at P<0.10.



Canola plants growing in glycerol amended soil without and with urea fertilizer added.

The available N levels in the glycerol amended treatments at the end of the experiment were not significantly different among treatments and were all low. This is consistent with N deficiency conditions that would be aggravated by the addition of a pure carbon substrate, with the plants using any available N that the microorganisms did not utilize. The 300 kg N ha<sup>-1</sup> urea treatment had significantly elevated nitrate as did the 100

and 1000 kg glycerol ha<sup>-1</sup> + urea treatments, indicating that there was more than sufficient N in these treatments for maximum yield. However, the 10000 rate had nitrate content that was not significantly different from the control and glycerol only treatments, indicating that N deficiency was limiting production in this treatment. The soil extractable P levels were not significantly affected by glycerol amendment, likely because the lack of N limited plant growth and P removal. Where urea was added along with glycerol, the extractable P contents were lower. There was little effect on K. Interestingly, the glycerol alone treatments resulted in significantly higher residual soil sulfate contents. The reason for this is not known, as it seems unlikely that the glycerol would add any significant amount of sulfate. As expected there was relatively little effect of the amendments on total N and total P. Some reductions in total P compared to the unfertilized, unamended control might be explained by removal in above ground harvested biomass. The effects of glycerol amendment on pH and EC were small and of little biological significance.

The addition of the highest rate of glycerol, with or without N, significantly increased soil organic carbon content compared to the controls. This is perhaps the most important effect, as glycerol addition may be of greatest benefit in increasing soil organic carbon content and carbon sequestration, compared to the alternative of incinerating the glycerol. The addition of glycerol at the highest rate of 10000 kg glycerol ha<sup>-1</sup> would increase soil C concentration by 2000 ug C g<sup>-1</sup> (4000 kg C ha<sup>-1</sup>) assuming that all the carbon added is conserved and not decomposed to carbon dioxide. Expressed on a carbon concentration basis in the soil (% organic carbon concentration) this would only result in an increase in the soil organic C % of 0.2% (i.e. 1.78 %C to 1.98%C), since 2000 ug C g<sup>-1</sup> = 0.2% C by weight. It is difficult to accurately detect such small increases from a single application. However, the detection of significant increases indicates that a significant portion of the carbon added as glycerol to the soil is still present in the soil after five weeks. Effects on residual metal content of the soil appear to be minor. The main noteworthy effect is an increase in residual extractable copper at the high rate of addition of glycerol, both without and with added nitrogen. This may reflect an enhancement in copper complexation and solubilization associated with increased concentration of organic acids formed during microbial decomposition of the glycerol.

Table 4.2 Soil properties in glycerol amendment trial before and after canola harvest.

Fertilizer Rate Glycerol or N kg ha <sup>-1</sup>	NO <sub>3</sub> -N ug/g	NH <sub>4</sub> -N ug/g	P ug/g	K ug/g	SO <sub>4</sub> -S ug/g	Total N mg/g	Total P mg/g	pH	EC mS cm <sup>-1</sup>	OC %	Cu ug/g	Zn ug/g	Cd ug/g
Before seeding	1.7	5.3	6.3	654	15.4	1.17	0.49	7.96	0.29	1.85	0.43	3.8	0.063
After harvest													
0	2.2c	6.9ab	5.6a	614a	12.0c	1.02bc	0.56ab	8.05a	0.30bcd	1.78d	0.44b	3.0a	0.058b
100	2.5c	6.9abc	5.7a	630a	12.0c	1.10abc	0.51b	7.95bc	0.30bcd	1.91cd	0.43b	2.6b	0.064ab
1,000	2.3c	6.0bc	5.2abc	618a	28.2b	1.11ab	0.61a	8.04a	0.32b	1.86cd	0.46b	2.9ab	0.069a
10,000	1.9c	5.9c	5.3ab	629a	42.1a	1.01c	0.53ab	7.90d	0.35a	2.18a	0.65a	2.9ab	0.064ab
0 + 300 (Urea)	10.5a	7.2a	4.8bcd	574b	11.0c	1.12a	0.54ab	7.98b	0.29bcd	1.85cd	0.44b	3.0a	0.062ab
100 + 300 (Urea)	9.4a	6.6abc	4.5cd	564b	14.3c	1.06abc	0.52ab	8.02a	0.29bcd	1.91cd	0.37b	3.1a	0.058b
1,000 + 300 (Urea)	5.6b	6.8abc	4.1d	577b	15.0c	1.12a	0.53ab	8.01ab	0.27d	1.98bc	0.42b	2.9ab	0.060b
10,000 + 300 (Urea)	2.8bc	7.1a	4.3d	623a	11.3c	1.14a	0.53ab	7.98b	0.28cd	2.10ab	0.58a	2.8ab	0.060b

Numbers in a column followed by the same letter are not significantly different at P<0.10

## Conclusions

- Alfalfa powders and pellets, ethanol production by-products including dry and wet distillers grain and thin stillage liquid are effective soil amendments as fertilizers to increase supplies of available nitrogen and other nutrients for plant growth. Low analysis compared to commercial inorganic fertilizers still makes them more costly to transport long distances and to apply.
- Availability of nitrogen from the solid amendments (alfalfa, DDG and WDG) **appears to be better than many other solid organic amendment sources such as manure and compost**, owing to a relatively high N content, especially for the distiller's grain products. Thin stillage is quite similar in properties and behavior to liquid swine manure but contains a better balance of N:S and N:P that is closer in line with crop requirements.
- No adverse alterations of soil conditions (salinity, pH, extractable metals) were observed resulting from the amendments as measured five weeks after addition. There were also no significant effects on enhanced metal uptake by the crop compared to commercial fertilizer amendment. However, some reduction of canola germination and emergence at the highest rates of WDG and DDG application tested was observed that merits further attention.
- Addition of glycerol to soil can increase soil organic carbon content, but must be supplemented by commercial fertilizer to account for the high nutrient immobilization potential when the glycerol is decomposed by the soil microbial population.
- Very high rates of glycerol addition (10,000 kg glycerol per hectare) appear to require unrealistically high rates of commercial fertilizer addition such as urea to provide enough nutrient for both microbial and plant growth. Rates of ~ 1000 kg glycerol per hectare may be more realistic as a field application rate.
- Evaluation of the performance of the amendments under field conditions is warranted.

## **PART 2: Soil Respiration, Nitrous Oxide Production and Nutrient Supply Rates Over a Ten Day Incubation**

### **Background/Introduction**

The objective of this component of the research was to assess how the amendments would affect important microbial processes in the soil. Following our study of the effects of the various amendments on canola growth and soil properties, their effects on production of carbon dioxide (a measure of microbial respiration and thereby microbial activity) and nitrous oxide (a greenhouse gas produced from microbial nitrification and denitrification) were determined in a ten day incubation. In the incubation, the release of available nutrients was assessed over the ten day period using Plant Root Simulator (PRS) probes.

The same treatments as described previously for the growth chamber experiments in Part 1 were prepared again in the same manner in pots. The pots were then brought to field capacity moisture content, and PRS resin membrane probes were installed to adsorb nitrate and phosphate released in the soil over the incubation period. The pots were then allowed to equilibrate and then were placed in sealed incubation chambers. Every two days for a period of ten days, samples of gas were removed from the headspace using a syringe. The gas samples were injected into vacutainers where they were stored until analysis of the carbon dioxide and nitrous oxide concentrations using a gas chromatograph.

### **Methods and Materials**

#### **Experimental Design**

The experiment was designed to include 19 treatments of soil organic amendments. The organic amendments included three rates of urea, alfalfa powder, wet distiller grains, thin stillage and glycerol, as used in the growth chamber experiments. The glycerol was applied with or without nitrogen (urea). A total amount of 800 g of soil was incubated in pots with a surface area of 113.04 cm<sup>2</sup>. Urea treatments included three rates: 0.0864, 0.1728 and 0.3456 g/pot, equivalent to 100, 200 and 400 kg N ha<sup>-1</sup>. Alfalfa pellets treatments were low rate: 1.5773; medium rate: 3.1564; and high rate: 6.3092 g/pot equivalent to 100, 200 and 400 kg N ha<sup>-1</sup>, respectively. Three rates of wet distillers grains were applied to soil; 4.36, 8.72 and 17.44 g/pot and 8.512, 17.024 or 34.048 g/pot of thin stillage were added to soil. As for the other treatments these low, medium and high rates represented a total N addition rate of 100, 200 and 400 kg N ha<sup>-1</sup>. The glycerol treatments included three rates with equivalency to 100 (low rate), 1000 (medium rate) or 10,000 kg glycerol ha<sup>-1</sup> (high rate) with or without 263.2 mg of urea (150 µg N g<sup>-1</sup> or 300 kg N / ha). A control that received no organic amendments was included. Each treatment was replicated four times.

#### **Treatment application**

A 650 g aliquot of soil was weighed first into each pot, and 50 g of soil was mixed with the amendment and spread on the soil surface on the pot. Then 150 ml of deionized water, which is required to bring soil moisture to field capacity level, was added and then 100 g of soil was placed on the top. In case of liquid or slurry amendments (glycerol, thin stillage) 700 g of soil was weighed into each pot, and then the amount of amendment was mixed well with 150 mL of deionized water and then added to soil. Then, 100 g of soil was placed on the top. All pots containing amended soil were placed on the bench in laboratory and remained in place for 6 hrs for stabilization.

#### **Incubation setup**

All the pots containing amended soil were placed into an airtight sealed container that was created from two PVC pipes 15 cm in diameter and 15 cm long with caps on each end. The two-part PVC container was joined together by a rubber airtight flange fastened with hose clamps. A rubber septum inserted into the cap was used to extract the gas samples. A 20-cm<sup>3</sup> syringe needle was used to collect the gas sample and transfer it into a 10-cm<sup>3</sup> evacuated vial. Sampling was done every two days at the same time of the day for 10 days. The incubation was carried out in an incubation chamber with electronically controlled environmental settings in which the chamber was set for 16 hr at 25 °C (day) and 8 hrs at 18°C (night). After each sampling time, the tops of the PVC containers were removed and allowed to remain open for 1 hr to allow natural airflow exchange between the chamber and the pots to ensure aerobic conditions. The collected gas was analyzed for CO<sub>2</sub> and N<sub>2</sub>O using gas chromatography. Due to limitations in number of PVC containers and space, the incubation was carried out in two sets. The first set of incubations included 10 treatments: three rates of urea, dehydrated alfalfa, and wet distillers grains in addition to a control treatment giving a total of 40 PVC containers. The second set of incubation included 12 treatments: three rates of glycerol with or without nitrogen, thin stillage and three rates of urea. Urea treatments were included for appropriate comparison in the two sets of incubations. A control that received no organic amendment was included as well.

#### **Nutrient availability measurement**

The bioavailable NO<sub>3</sub>-N, NH<sub>4</sub>-N, and PO<sub>4</sub>-P supply rates in the soil were determined using Plant Root Simulator (PRS<sup>TM</sup>) resin membrane probes as ion sinks. The PRS<sup>TM</sup> anion probes were initially soaked into distilled water for 24 hrs. Then, the probes were charged for 2 hrs in 0.5 M NaHCO<sub>3</sub> (baking soda) to saturate the exchange sites with bicarbonate as the counter ion, and this was repeated 4 times. The probes were then washed twice and stored in distilled water until they were inserted into the soil. PRS<sup>TM</sup> cation probes were charged by soaking in 0.5 M HCL two times for 2 hrs of each to saturate the exchange sites with H<sup>+</sup> ions. Then, the anion and cation probes were inserted into the pots containing amended soil and remained installed for 10 days. At the end of incubation, the probes were removed from the soil and placed into plastic Ziplock<sup>TM</sup> bags and transported to the laboratory. Then, the probes were washed properly to remove all remaining soil particles and placed into a clean Ziplock<sup>TM</sup> bag in which each probe was placed in a separate Ziplock<sup>TM</sup> bag. A 20-ml of 0.5 M HCl was added to each bag containing the probe and remained for one hour to elute the sorbed ions from the membrane surface. The eluent was then placed in a 7 dram vial, capped, and stored at 4°C until it was colorimetrically analyzed for NO<sub>3</sub>-N, NH<sub>4</sub>-N and PO<sub>4</sub>-P using Technicon<sup>TM</sup> Auto-Analyzer II.



Placing PRS probes in amended soil in pots (left), and pots in incubation chambers (right).



## Results and Discussion

### Carbon Dioxide Evolution (Soil Respiration) $\mu\text{g CO}_2 \text{ cm}^{-2} \text{ hr}^{-1}$

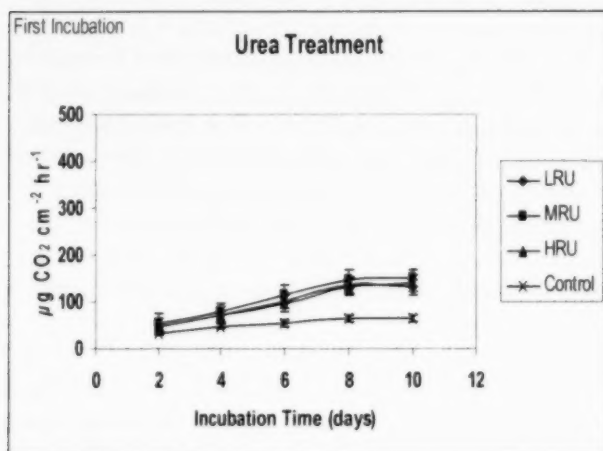


Fig. 1 Effect of application of three rates of urea: high rate (HR); medium rate (MR); low rate (LR) on  $\text{CO}_2$  evolution from soil during the first set of incubation.

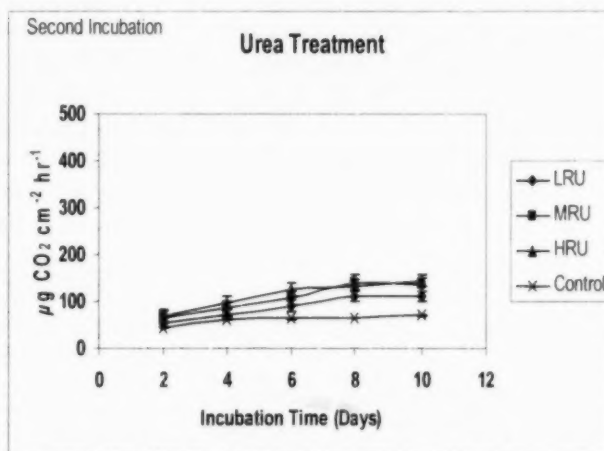


Fig. 2 Effect of application of three rates of urea: high rate (HR); medium rate (MR); low rate (LR) on  $\text{CO}_2$  evolution from soil during the second set of incubation.

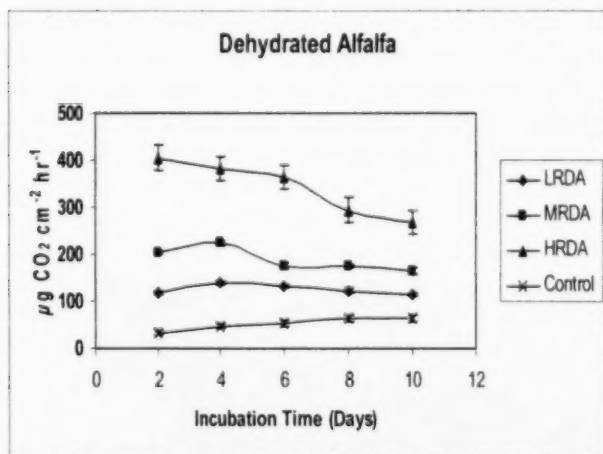


Fig.3 Carbon dioxide evolved from soil amended with three rates of dehydrated alfalfa: high rate (HR); medium rate (MR) and low rate (LR).

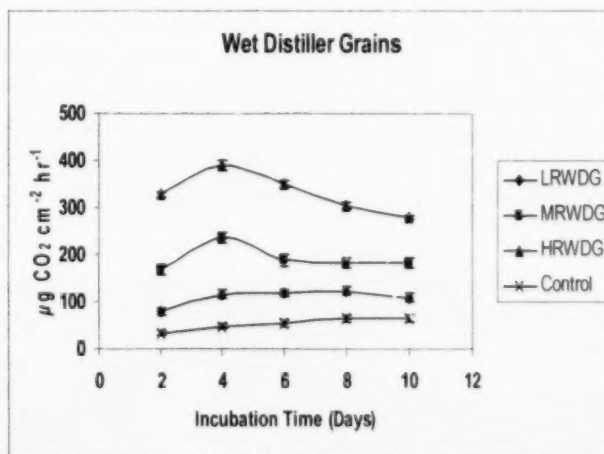


Fig. 4 Carbon dioxide evolved from soil amended with three rates of wet distillers grains: high rate (HR), medium rate (MR) and low rate (LR).



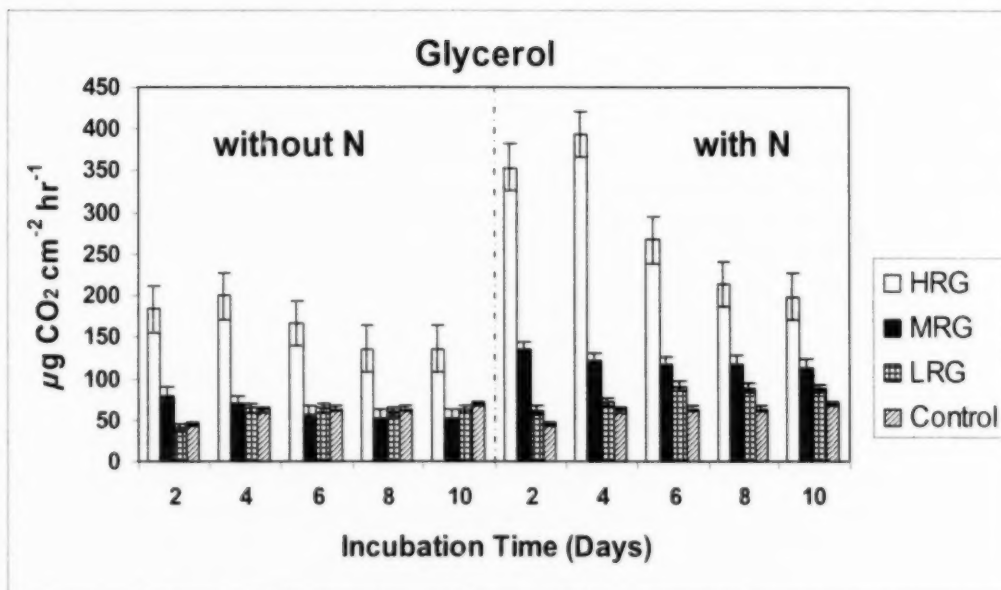


Fig. 5 Carbon dioxide evolved from soil amended with three rates of glycerol: high rate (HR), medium rate (MR) and low rate (LR) with or without N.

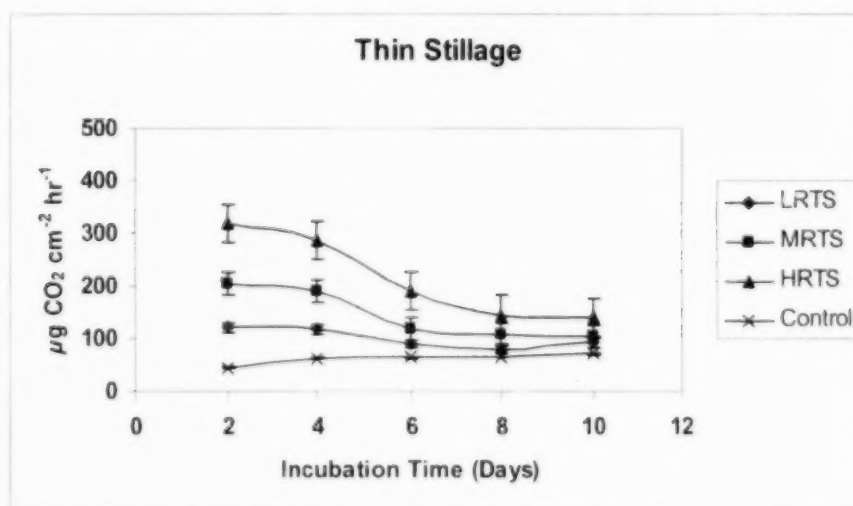


Fig. 6 Carbon dioxide evolved from soil amended with three rates of thin stillage: high rate (HR), medium rate (MR) and low rate (LR).

Amendment with urea stimulated microbial activity in the soil, as shown by significantly higher rates of carbon dioxide production in the urea amended soils than the control in both incubation experiments (Figures 1 and 2). The effect was delayed, with CO<sub>2</sub> evolution not reaching a peak until days 8 and 10. This CO<sub>2</sub> is likely derived from enzymatic (urease) hydrolysis of the urea to CO<sub>2</sub> and ammonia, as well as stimulation of heterotrophic microbial activity. All of the organic amendments stimulated microbial activity in the soil, with elevated CO<sub>2</sub> production compared to the unamended control (Figures 3-6). There were generally higher fluxes of CO<sub>2</sub> in the N containing amendment treatments than urea at equivalent rates of added N, especially for the medium and high amendment rates. The rate effect was much more apparent for the organic amendments than for urea, and reflects the effect of the addition of substrate carbon for microbial decomposition along with nitrogen in the amendment treatments. Generally, fluxes of CO<sub>2</sub> were greatest for the amendments at the two day measurement period and declined thereafter, presumably related to microbial consumption of the amendment. The decrease in CO<sub>2</sub> evolution from the soil with time was greater in the thin stillage treatment (Fig. 6) compared to other amendments. This can be explained by more of the organic carbon in the thin stillage being present in soluble, low molecular weight forms that would undergo more rapid decomposition and depletion. The dehy alfalfa and wet distillers grain resulted in the greatest CO<sub>2</sub> evolution per unit of N added, owing to a higher carbon content relative to N than thin stillage or urea. CO<sub>2</sub> flux from glycerol amended soil was low compared to other amendments, due to lack of nitrogen restricting microbial activity. When N fertilizer was added, carbon dioxide evolution rates were significantly increased, as the N fertilizer supplied the N needed for microbial growth.

## Nitrous Oxide (N<sub>2</sub>O) Evolution $\mu\text{g N}_2\text{O cm}^{-2} \text{hr}^{-1}$

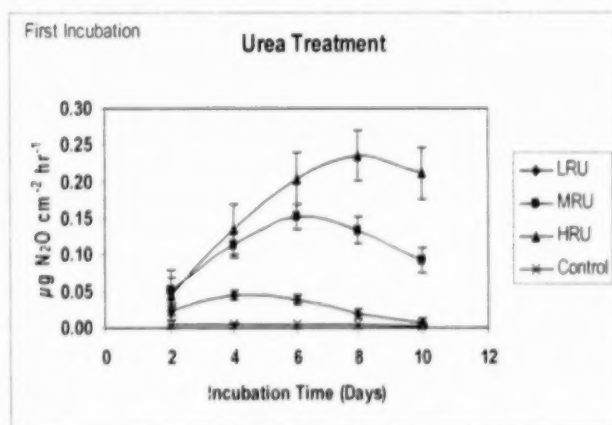


Fig. 7 Effect of application of three rates of urea: high rate (HR); medium rate (MR); low rate (LR) on N<sub>2</sub>O evolution from soil during the first set.

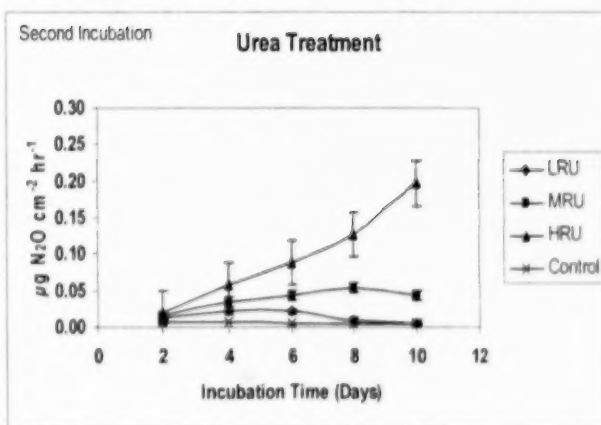


Fig. 8 Effect of application of three rates of urea: high rate (HR); medium rate (MR); low rate (LR) on N<sub>2</sub>O evolution from soil during the second set.

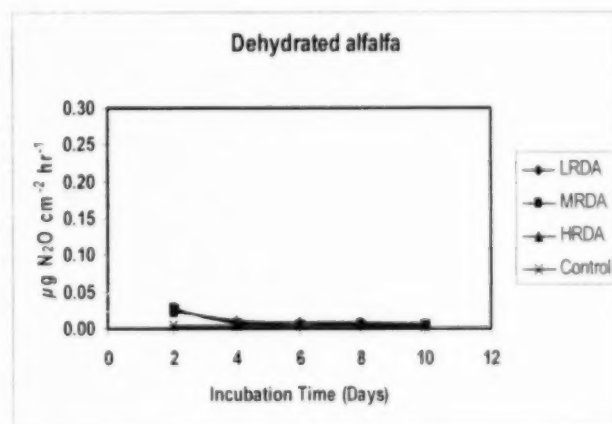


Fig. 9 Nitrous oxide evolved from soil amended with three rates of dehydrated alfalfa : high rate (HR); medium rate (MR) and low rate (LR).

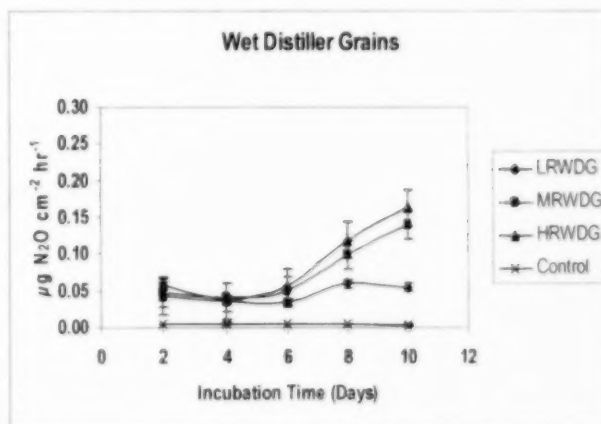


Fig. 10 Nitrous oxide evolved from soil amended with three rates of wet distillers grain: high rate (HR), medium rate (MR) and low rate (LR).

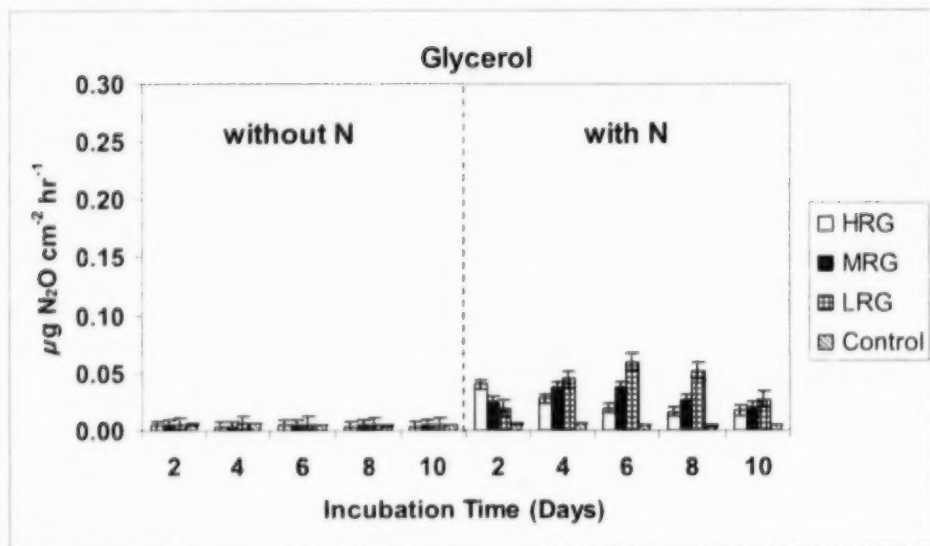


Fig. 11 Nitrous oxide evolved from soil amended with three rates of glycerol; high rate (HR), medium rate (MR) and low rate (LR) with or without N.

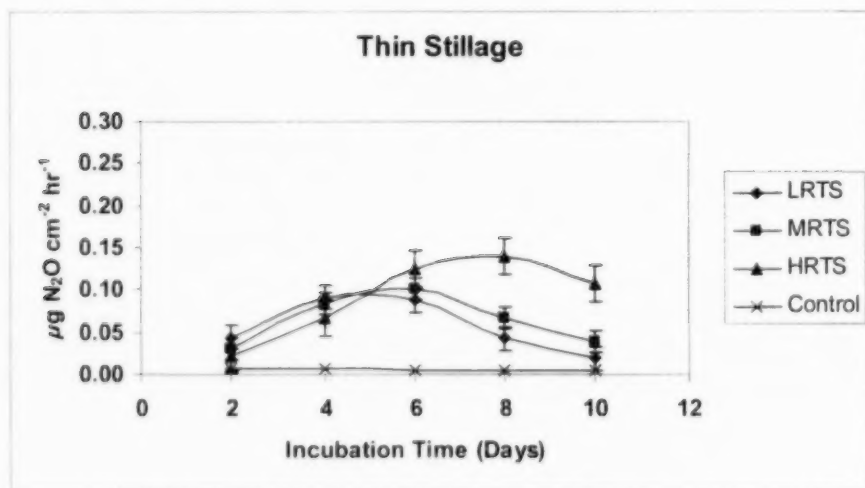


Fig. 12 Nitrous oxide evolved from soil amended with three rates of thin stillage; high rate (HR), medium rate (MR) and low rate (LR).

Rates of evolution of  $\text{N}_2\text{O}$  were about 1000 times less than for  $\text{CO}_2$  over the ten days of the incubation. Fluxes of  $\text{N}_2\text{O}$  from the soil surface generally increased over the first few days of the incubation, followed by a leveling off or decrease (Fig. 7 and 8). Rates of  $\text{N}_2\text{O}$  production were the highest and sustained over the longest period at the high rate of urea addition, as expected. As the nitrate content of the initial soil was quite low, and the moisture content was at field capacity or less, it would appear that the  $\text{N}_2\text{O}$  evolution observed over this time period is originating from the nitrification process. Of the organic amendments, the wet distillers grain and thin stillage produced the highest rates of  $\text{N}_2\text{O}$  production per unit of N added (Figs. 10 and 12). This can be attributed to a greater net release of ammonium by mineralization, due to a narrow C:N ratio and more easily decomposed organic materials, to produce ammonium that was subsequently nitrified to nitrate, producing  $\text{N}_2\text{O}$ . Of the N containing amendments, the dehydrated alfalfa produced low amounts of  $\text{N}_2\text{O}$ , with total production of  $\text{N}_2\text{O}$  over the ten days that was significantly less than wet distillers grain or thin stillage. As expected, the glycerol without added N resulted in very little  $\text{N}_2\text{O}$  produced (Figure 11), since immobilization is not anticipated to be associated with nitrous oxide production. The treatments where urea N fertilizer was added with the glycerol resulted in elevated  $\text{N}_2\text{O}$  production, but levels were still low compared to wet distillers grain and thin stillage, and closer to flux rates observed for dehydrated alfalfa.

Soil Nutrient Supply Rates:  $\mu\text{g}$  nutrient sorbed per  $\text{cm}^2$  of membrane over 10 days.

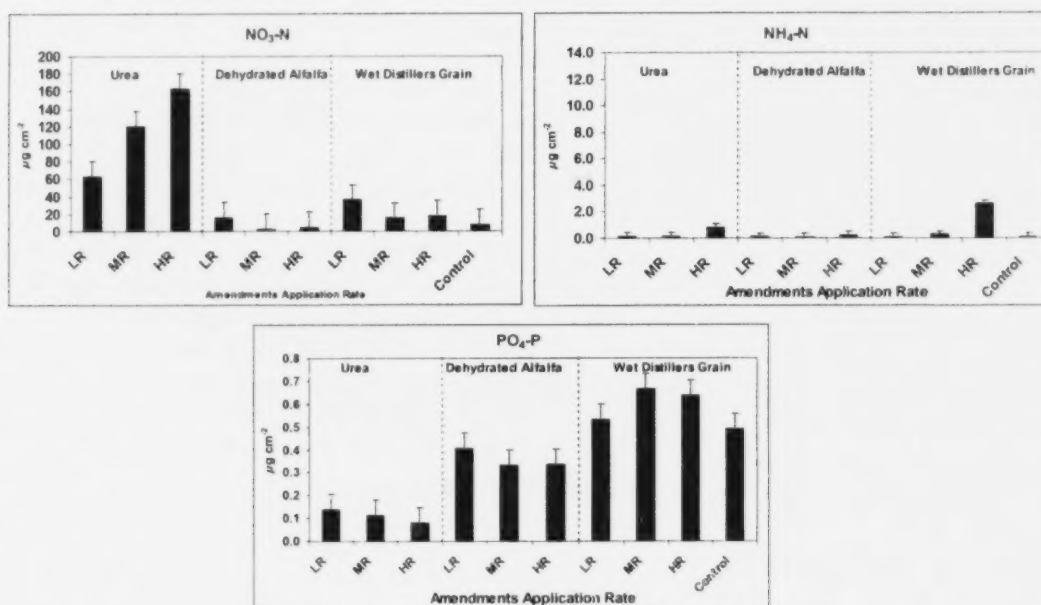


Fig.13 Nutrient supply rate (  $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$ ) in soil amended with different rates (high rate: HR; medium rate: MR and low rate: LR) of different organic materials in first incubation set.

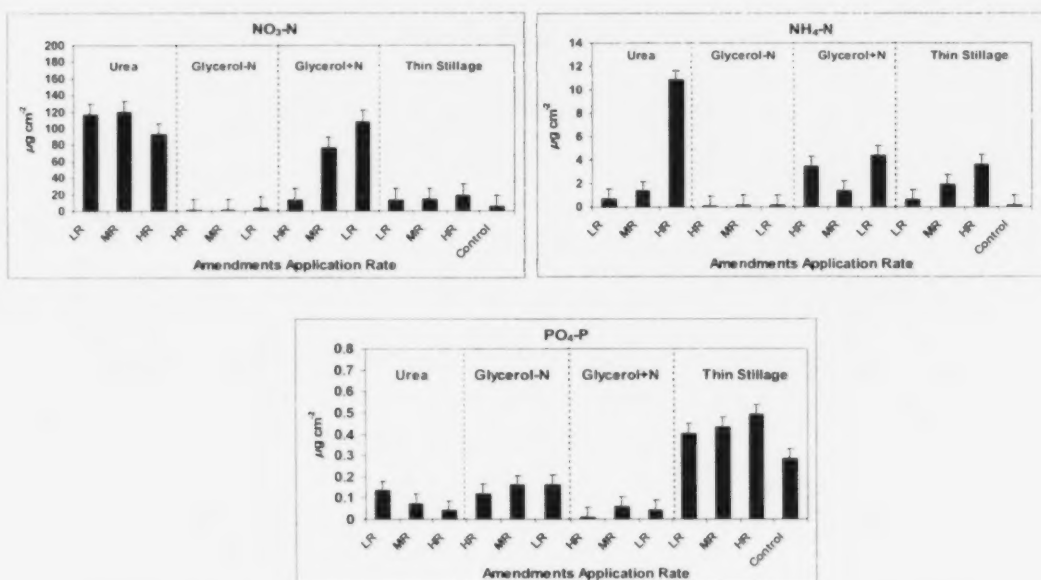


Fig.14 Nutrient supply rate ( $\text{NO}_3\text{-N}$ ,  $\text{NH}_4\text{-N}$  and  $\text{PO}_4\text{-P}$ ) in soil amended with different rates (high rate: HR; medium rate: MR and low rate: LR) of different organic materials in the second incubation set



The supply rates of nitrate to the PRS (Plant Root Simulator) resin membrane probe over the ten day period (Figures 13 and 14) were closely related to the patterns in  $N_2O$  production. The greatest supply rates of nitrate were observed in the high rate ( $400 \text{ kg N ha}^{-1}$ ) urea treatment, and this was the treatment with the greatest production of  $N_2O$ . Ammonium supply rates were generally low for the low and medium rate urea treatments, indicating that ammonium formed from urea hydrolysis was rapidly converted to nitrate in the nitrification process. Nitrification is likely the dominant mechanism and source of nitrous oxide in this incubation experiment. Some elevation in ammonium supply rates was noted in high rates of some of the amendments like wet distillers grain and thin stillage. This likely reflects a higher content of ammonium present initially in these amendments compared to other amendments like alfalfa and urea, in which ammonium is formed by decomposition or hydrolysis. The effect of adding dehydrated alfalfa and wet distillers grains on the supply rate of nitrates over ten day of the incubation was limited (Fig 13), with only small increases in the case of wet distillers grain, or decreases in the case of dehydrated alfalfa. This indicates that the release of available nitrogen from these amendments in the first few days was limited by some microbial immobilization, likely by a C:N ratio that was higher in the case of alfalfa than wet distillers grain. This also corresponded with low  $N_2O$  emission rates from the alfalfa amendment. More available N, and  $N_2O$ , would likely be released in following weeks as microbial decomposition of the amendments proceeded and C:N in the soil narrows. A greater release of available N over 5 weeks is indicated by a significant rate effect on increasing canola N uptake. The limited effect on available N supply measured by PRS over the first ten days indicates an initial delayed release of available N from these amendments.

The effect of the glycerol amendment alone on immobilizing nearly all soil available N is evident in the very low supply rates of nitrate and ammonium in these treatments (Fig 14 ). Addition of urea with the glycerol increased the supply rates of ammonium and nitrate, especially at the low rates of glycerol addition. The effect of increasing rate of glycerol addition on increasing immobilization is evident in lower supply rates of nitrate with increasing rate of glycerol. There appears to be sufficient available N provided with the amendment of  $300 \text{ kg N ha}$  of urea to meet microbial and plant demands at the low and medium rates of glycerol addition.

As expected, urea addition reduced soil phosphate supply rates over the ten day incubation, likely as a result of microbial utilization/immobilization of soil P as a result of stimulation of growth by the addition of N. Of the organic amendments, the wet distillers grain and thin stillage were most effective at increasing soil supply rates of available P (Figures 13 and 14). The addition of glycerol, especially when combined with urea, resulted in large decrease in soil phosphate supply rate. This is attributed to microbial immobilization of phosphate during the decomposition of glycerol, which would be further promoted by removal of N limitations through addition of urea. These results indicate that P deficiency and a need for supplemental P fertilization would also be an important consideration when adding glycerol to soils.

## Conclusions

- Per unit of N added, the dehydrated alfalfa and wet distillers grain resulted in the greatest evolution of carbon dioxide from the soil. This is explained by these amendments containing the largest amounts of carbon per unit of nitrogen added that acts as substrate for microbial decomposition. The stimulation of microbial activity by thin stillage was less and of a shorter duration, due to less carbon added that was more easily decomposed compared to the other organic amendments.
- Addition of nitrogen fertilizer along with glycerol enhances microbial activity and decomposition.
- Per unit of N added, urea tended to result in the greatest production of  $N_2O$ , followed by wet distillers grain and thin stillage, with glycerol and dehydrated alfalfa resulting in the lowest nitrous oxide production. The observed differences in soil supply rates of nitrate to the PRS probe as affected by source and rate were closely related to the patterns in  $N_2O$  produced over the ten day incubation, with highest supply rate of nitrate and  $N_2O$  production observed for urea. It appears that nitrification of ammonium contained or produced by the amendment addition is the main source of  $N_2O$  produced over the ten days of the incubation.
- Considering the good plant response to the addition of the alfalfa powder observed in the growth chamber experiments in Part 1, plant productivity per unit of greenhouse gas (carbon dioxide plus nitrous oxide) produced may be anticipated to be the greatest with this amendment.
- With the exception of urea and glycerol, the organic amendments generally increased soil phosphate supplies. Decrease in soil phosphate supply from glycerol addition indicates that phosphate fertilization would also benefit microbial growth and stabilization of carbon derived from glycerol amendment.

## **General Conclusions and Recommendations**

All the organic amendments tested appear to have potential value as a means to increase soil fertility, soil organic matter content and carbon sequestration. These organic matter amendments with high nitrogen content relative to carbon content (C:N ratio less than 20:1) such as the dehydrated alfalfa, distillers grain, and thin stillage were quite effective at releasing available nitrogen to the crop over the five week period evaluated. The amendments dehydrated alfalfa, distillers grains and stillage resulted in significant increases in nutrient uptake that were as good or better than have been reported with many manures and composts in previous studies. Some inhibitory effects on canola germination at the highest rates of distiller's grain application suggests that caution should be used when applied at high rates, and further evaluation of the nature of the inhibition is warranted. There were no adverse effects observed on soil chemical properties (salinity, pH) or available metal contents in the soil or the plant material with any of the organic amendments at the rates tested. Glycerol amendment can increase soil organic carbon, but nitrogen fertilizer must be added to provide sufficient available nitrogen to meet both plant and microbial requirements during the decomposition. An alternative is to grow a legume such as pea on glycerol amended soil that can compensate for the immobilization of soil N by fixing its own N through rhizobia symbiosis.

Addition of the organic amendments to the soil stimulated microbial activity and respiration in line with the amount and composition of the organic matter added. Solid amendments such as distillers grain and alfalfa powder were more effective than thin stillage in promoting a sustained increase in microbial respiration. Production of nitrous oxide per unit of N added was lowest with the alfalfa powder, suggesting that this amendment may have the lowest impact on increasing greenhouse gas production when added to the soil. Soil nitrate supply rate to PRS probes was closely related to patterns in nitrous oxide production observed among the amendments. Very low soil supply rates of phosphate in glycerol amended soil indicate that glycerol amended soils would benefit from both supplemental fertilizer nitrogen and phosphorus to ensure adequate nutrient for microbial and plant growth.

Testing of the performance of these amendments under field is warranted, and field trials with thin stillage have started in the fall of 2008.

## **Acknowledgements**

Acknowledgement given to Agriculture Development Fund at the end of papers and presentations incorporating material from this study.

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## **Equipment**

None

## **Project Developed Materials**

### **Conference Presentations with Published Proceedings**

J.J. Schoenau, 2007. Potential Impacts of Biofuel Production on Soils. Proceedings of Capturing Feed Grain and Forage Opportunities: Farming for Feed, Forage and Fuel Conference, December 11-12, Red Deer, Alberta, 119-123.

F. Al Otaibi and J.J. Schoenau, 2008. The Effect of Glycerol as an Organic Soil Amendment on the Growth of Plants. Proceedings of the 2008 Soils and Crops Workshop, February 28-29, Saskatoon, Saskatchewan. (on CD).

P. Qian and J.J. Schoenau, 2008. Effect of Soil Amendment with Alfalfa Pellets and Glycerol on Nutrition and Growth of Wheat. Proceedings of the 2008 Soils and Crops Workshop, February 28-29, Saskatoon, Saskatchewan, (on CD).

J.J. Schoenau, 2009. Potential Impacts of Biofuel Production on Soils: Fertilizer Value of Biofuel By-Products. IN: Proceedings of Saskatchewan Soil Conservation Association 21<sup>st</sup> Annual Conference: Feeding and Fueling the World. February 11 and 12, 2009, Saskatoon, Saskatchewan.

### **Popular Press Coverage**

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